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BIOREMEDIATION OF HD-CONTAMINATED SOIL

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RESEARCH AND TECHNOLOGY DIRECTORATE

December 1996

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DEPARTMENT OF THE ARMY
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PREFACE

The work described in this report was authorized under the Alternative Technology Program. This work was started in March 1996 and completed in September 1996.

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BIOREMEDIATION OF HD-CONTAMINATED SOIL

1. INTRODUCTION

Many current U.S. government installations and formerly used defense sites are known to contain buried chemical warfare materials. HD (sulfur mustard, 2,2'-dichlorodiethyl sulfide, CAS # 505-60-2) is among the most commonly found of these materials¹. Remediation work at these sites is in the initial stages and there is no commonly accepted HD remediation technology. Excavation followed by incineration is one potential approach but remediation of these sites is expected to take many years and it is not certain that the incineration option will remain open in future years. Since transportation of chemical warfare materials is also a very sensitive issue, onsite treatment may be required. Bioremediation generally offers the advantage of economical and low-temperature operation and is currently in use for many environmental contaminants. However, bioremediation has not previously been demonstrated for HD contaminated soil. The present study illustrates one approach to HD soil bioremediation. Because HD is not expected to be biodegradable due to its aqueous insolubility² and very high toxicity to microorganisms³ it was necessary to first hydrolyze the HD in the soil. This was accomplished with a 90 minute reaction with hot water. It has previously been demonstrated that liquid HD (not within a soil matrix) can be hydrolyzed in hot water primarily to thiodiglycol which is readily biodegradable⁴. The purpose of this study was to demonstrate an experimental system in which soil could be homogeneously spiked with HD and in which the hydrolysis of the HD and the biodegradation of its products could be monitored. The HD used for this study was a mixture of aged HD (64.2% pure) which had been recovered from an actual burial site (Spring Valley, Washington D.C.) and [¹⁴C] radiolabeled HD which was used for tracking the radioactivity within the soil/water/biomass matrix in the bioreactor as well as the evolution of [¹⁴C]CO₂ resulting from the biological mineralization of the HD hydrolysis products.

2. MATERIALS AND METHODS

2.1. HD

HD-3035-CTF-N-SCBRD-RTL-SH was obtained from a 75 mm projectile excavated from the Spring Valley burial site in Washington D.C. in 1993, where it had presumably been buried for approximately 75

years. It was 64.2 area % pure by nuclear magnetic resonance spectroscopy; the main impurities were HD disulfide ($\text{ClCH}_2\text{CH}_2\text{S}$)₂ - 11.8 area %) and 1,4-dithiane (9.7 area %)⁵.

2.2. [¹⁴C] HD

[¹⁴C] HD was synthesized by Illinois Institute Technology Research Institute and was 98.7 % pure by Gas Chromatography (GC). Carbons were uniformly labeled and the specific activity was 5 mCi/mmol.

2.3. Synthetic soil

Synthetic soil consisted of 10% peat, 20% clay, 69% sand and 1% lime.

2.4. Bioreactor Sampling

Bioreactor Sampling was done as follows. For the NaOH CO₂ trap, 100 ul was withdrawn and replaced with 100 ul of distilled water to maintain a constant volume. The NaOH sample was added to 3 ml of distilled water and adjusted to a pH of 11 using HCl. This prevented the formation of precipitate which interfered with scintillation counting. Soil samples were removed at the same time points as NaOH samples. One ml of slurry was withdrawn from the reaction vessel and replaced with one ml of water. The 1 ml sample was added to 19 ml of scintillation cocktail and shaken to ensure that a consistent suspension formed. This suspension was quickly diluted 1:10 in scintillation cocktail to avoid scintillation counting interference caused by high concentrations of soil in the cocktail.

2.5. Scintillation counting

Scintillation counting was performed by adding either 1 ml of bioreactor slurry or 0.1 ml neutralized (pH 11.0) NaOH to a 20 ml scintillation vial. The vial was filled with 20 ml of Ready Safe liquid scintillation cocktail (Beckman, Fullerton, CA) and counted in a Packard 1900 TR Liquid Scintillation Analyzer. Counting efficiency was approximately 95%.

2.6. Modified Wolin Salts Solution

Modified Wolin Salts Solution was prepared 100x as follows: 3.0 g/L nitrilotriacetic acid, 6.0 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L MnSO_4 , 0.5 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/L $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g/L H_3BO_3 , 0.01 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 0.01 g/L $\text{MoO}_4 \cdot 2\text{H}_2\text{O}$.

3. RESULTS AND DISCUSSION

3.1. Soil Spiking

The excavated Spring Valley HD was selected as a "worst-case" substrate and was mixed with [^{14}C] radiolabeled HD in a ratio of 3.3 g to 38.1 mg. This material was added to 500 g of synthetic soil and blended in a Waring commercial blender at low speed for one hour with occasional scraping of the sides of the vessel. The resulting soil/HD mixture was tested for homogeneity of the HD distribution by withdrawing five samples which were analyzed for radioactivity in a scintillation counter. Results (Table 1) show that the counts per minute (CPM) of radioactivity per milligram (mg) of soil was consistent within a standard deviation of 12.4%.

Sample	Total CPM	mg soil	CPM/mg	Statistics
1	9873.9	39.1	252.5	Average = 221.1 CPM/mg soil
2	9252.7	37.9	244.1	Standard Deviation = 27.4 CPM/mg soil
3	12574.1	54.8	229.5	% Standard Deviation = 12.4
4	11073.1	61.6	199.8	
5	10469.6	52.4	221.1	

Table 1. Homogeneity of radiolabeled HD within soil matrix.

3.2. Hydrolysis

Spiked soil (150 g) was mixed with 300 g of water, heated to 90° C, and agitated with a stir bar in a 250 ml Erlenmeyer flask for 90 minutes. This material was headspace analyzed for HD by gas chromatography/mass spectroscopy to a level of 200 parts per billion (ppb). This level represents the U.S. Army military drinking water standard for HD. No HD was detected at or above the 200 ppb level.

3.3. Biodegradation

The soil slurry containing the hydrolysis products was

divided into three portions of approximately equal volume and added to 250 ml Erlenmeyer flasks which were used as reactors. The setup is illustrated in Figure 1.

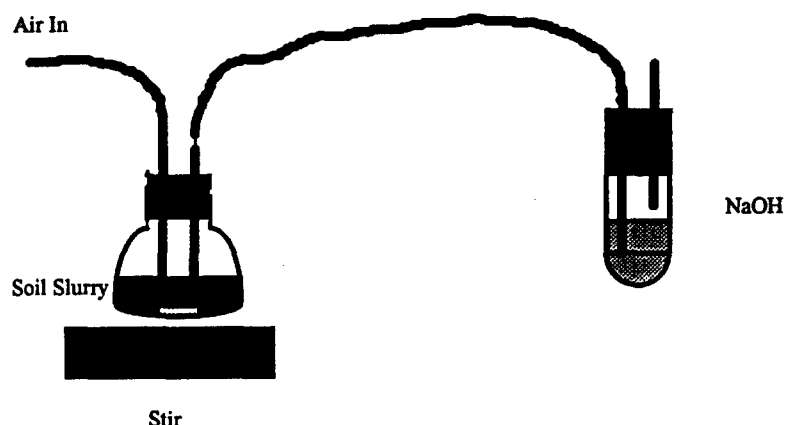


Figure 1 Soil slurry bioreactor apparatus with CO₂ trap (5M NaOH).

Hydrolyzed, spiked soil was amended with 4 g mixed liquor suspended solids (MLSS) activated sludge per g HD (29.3 g of 0.6 g/L MLSS), 10 ml/L modified Wolin Salts trace inorganic salts solution, 0.18 g NH₄Cl per g HD, 0.033 g KH₂PO₄ per g HD and 3.5 g NaHCO₃ per g HD (for pH control). Air was continuously provided to the reactors through a peristaltic pump. Exhaust air was purged through 30 ml of 5 M NaOH to trap [¹⁴C]CO₂ for subsequent analysis of radioactivity levels. Reactors were agitated with a stir bar and the sides were regularly washed down to prevent the accumulation of solids on the sides of the flask. Figure 2 illustrates the decrease in radioactivity in the slurry and the resultant increase in radioactivity recovered from the NaOH CO₂ trap. The recovery of [¹⁴C]CO₂ from [¹⁴C]HD provides direct evidence of the biological mineralization of the hydrolyzed product. Not all the radioactivity is converted to CO₂ since carbon is also diverted to new biomass and to other biological degradation products.

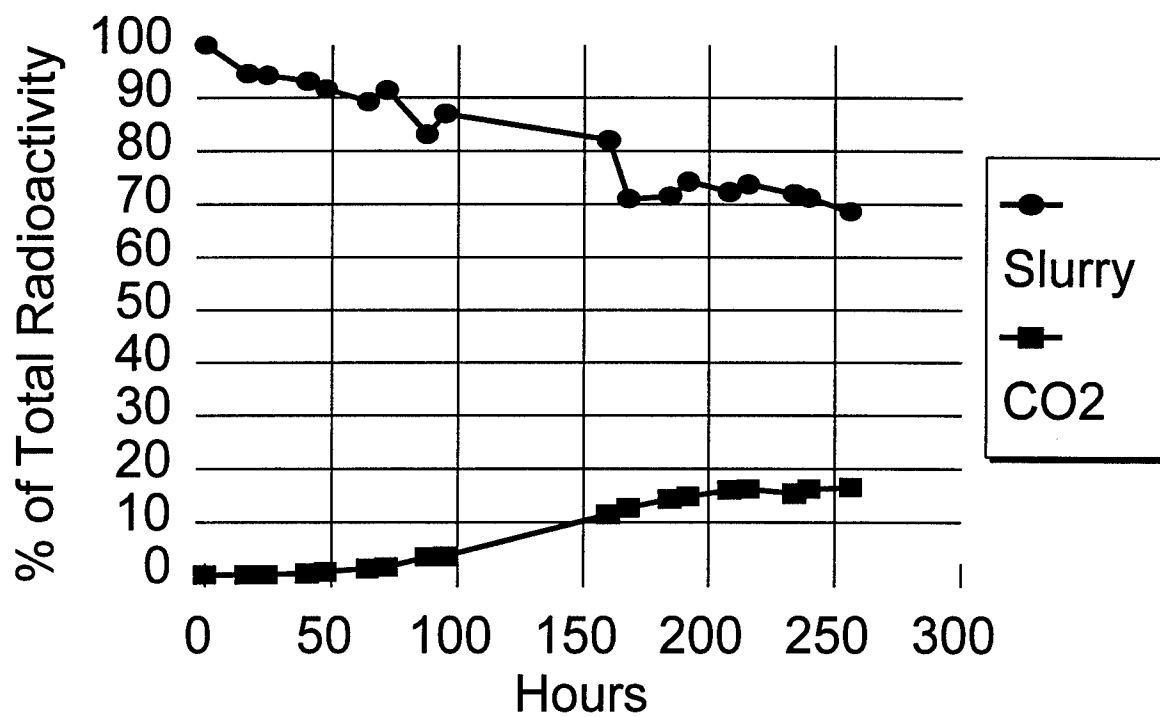


Figure 2. Mineralization of $[^{14}\text{C}]\text{HD}$ from soil: percent of radioactivity from CO_2 and from soil slurry as a function of reaction time.

The overall average recovery of radioactivity was defined by equation (1):

$$\frac{\text{CPM in original slurry}}{\text{CPM in slurry @ time} + \text{CPM in NaOH CO}_2 \text{ trap @ time}} \quad (1)$$

For the three reactors used in the experiment, illustrated in Figure 2, the average recovery of radioactivity was 89.9%. In general, the efficiency of recovery decreased with time, likely due primarily to sampling losses. The efficiency of recovery as a function of time is illustrated by Figure 3.

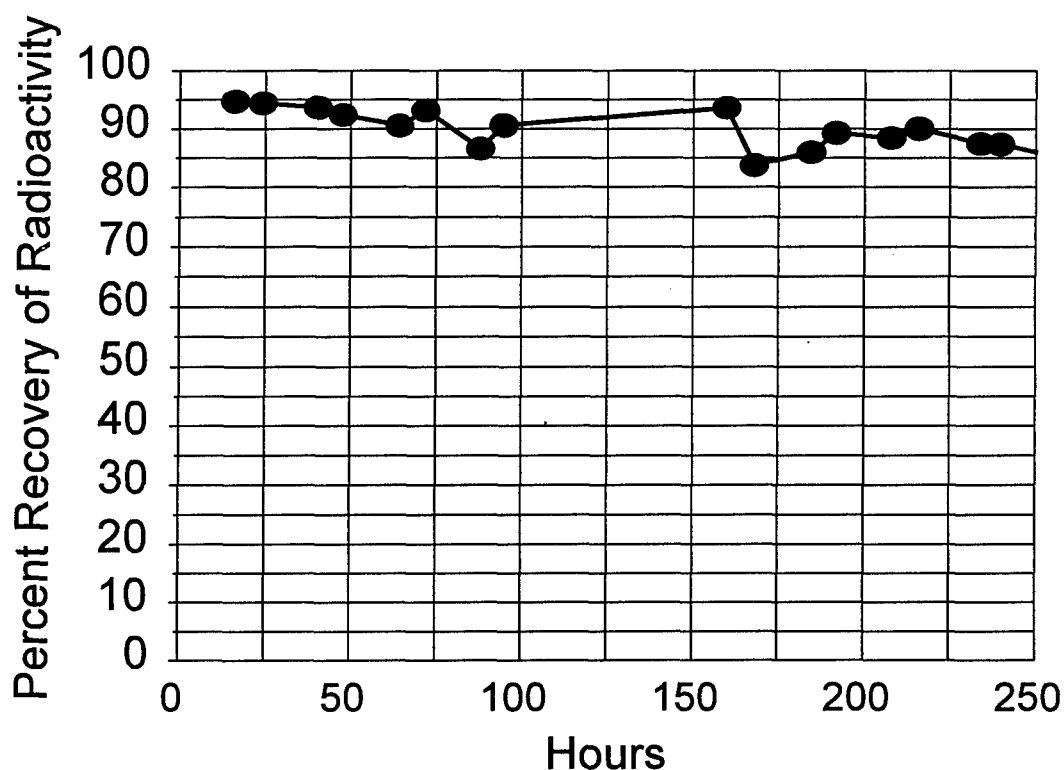


Figure 3. Percent recovery of radioactivity as a function of reaction time.

4. CONCLUSIONS

The experimental system described here demonstrates that HD can be spiked into an artificial soil matrix, distributed in a reasonably homogeneous manner ($\pm 12.4\%$) by agitation in a blender, hydrolyzed in 90° C water to HD levels below 200 ppb within 90 minutes, and biologically mineralized by the addition of activated sludge and inorganic salts. Results suggest a potential application of this approach to the environmental remediation of HD contaminated soil.

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APPENDIX A.

Reactor #1: Samples from NaOH trap (100 ul each)

Hours	CPM	Corrected	CPM per 30 mL	CPM/mg soil	% starting CPM
0	14.40	0.00	0.00	0.00	0.00
16.5	21.20	6.80	2040.00	0.09	0.02
24.5	33.90	19.50	5850.00	0.25	0.06
40.5	94.30	79.90	23970.00	1.04	0.25
48	144.10	129.70	38910.00	1.69	0.40
64.5	265.00	250.60	75180.00	3.26	0.77
72	251.80	237.40	71220.00	3.09	0.73
88	740.20	725.80	217740.00	9.44	2.24
95	745.40	731.00	219300.00	9.51	2.26
160	2802.60	2788.20	836460.00	36.27	8.61
168	3190.30	3175.90	952770.00	41.32	9.81
184.5	4091.10	4076.70	1223010.00	53.04	12.59
192	4030.50	4030.50	1209150.00	52.43	12.45
208	4832.40	4382.40	1314720.00	57.01	13.53
216	4734.30	4734.30	1420290.00	61.59	14.62
234	4343.80	4343.80	1303140.00	56.51	13.41
240	4989.70	4989.70	1496910.00	64.91	15.41
256.5	5031.90	5031.90	1509570.00	65.46	15.54

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APPENDIX B.

Reactor #1: Soil slurry samples (1 ml each)

Time	Corrected CPM	CPM per sample	CPM/mg soil	% of starting CPM
0.0	9676.90	9712704.53	421.19	100.00
16.5	8810.50	8843098.85	383.48	91.05
24.5	9100.00	9133670.00	396.08	94.04
40.5	8539.20	8570795.04	371.67	88.24
48.0	8906.30	8939253.31	387.65	92.04
64.5	8773.00	8805460.10	381.85	90.66
72.0	9117.90	9151636.23	396.86	94.22
88.0	7592.60	7620692.62	330.47	78.46
95.0	8789.50	8822021.15	382.57	90.83
160.0	8095.10	8125051.87	352.34	83.65
168	7230.80	7257553.96	314.72	74.70
184.5	7280.40	7307337.48	316.88	75.22
192	7321.20	7348288.44	318.66	75.64
208	7206.40	7233063.68	313.66	74.45
216	7310.30	7337348.11	318.19	75.53
234	7104.20	7130485.54	309.21	73.40
240	6664.30	6688957.91	290.07	68.85
256.5	6805.10	6830278.87	296.20	70.31

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APPENDIX C.

Reactor #2: Samples from NaOH trap (100 ul each)

Time	CPM	Corrected CPM	CPM per 30 ml	CPM/mg soil	% starting CPM
0.00	6.10	0.00	0.00	0.00	0.00
16.50	46.40	40.30	12090.00	0.54	0.13
24.50	86.50	80.40	24120.00	1.08	0.25
40.50	187.90	181.80	54540.00	2.43	0.57
48.00	264.30	258.20	77460.00	3.46	0.81
64.50	558.40	552.30	165690.00	7.39	1.74
72.00	714.90	708.80	212640.00	9.49	2.23
88.00	1306.30	1300.20	390060.00	17.41	4.09
95.00	1302.50	1296.40	388920.00	17.35	4.08
160.00	3942.60	3936.50	1180950.00	52.70	12.39
168.00	4305.80	4299.70	1289910.00	57.56	13.53
184.50	4837.40	4831.30	1449390.00	64.68	15.21
192.00	4856.90	4856.90	1457070.00	65.02	15.29
208.00	4992.50	4992.50	1497750.00	66.83	15.71
216.00	5304.70	5304.70	1591410.00	71.01	16.70
234.00	4890.20	4890.20	1467060.00	65.46	15.39
240.00	5081.90	5081.90	1524570.00	68.03	16.00
256.50	4810.70	4810.70	1443210.00	64.40	15.14

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APPENDIX D.

Reactor #2: Soil slurry samples (1 ml each)

Time	Corrected CPM	CPM per spl	CPM/mg soil	% of starting CPM
0.00	9686.30	9529381.94	425.23	100.00
16.50	9302.70	9151996.26	408.39	96.04
24.50	9134.90	8986914.62	401.02	94.31
40.50	9073.40	8926410.92	398.32	93.67
48.00	8143.20	8011280.16	357.49	84.07
64.50	8345.50	8210302.90	366.37	86.16
72.00	8341.50	8206367.70	366.19	86.12
88.00	8165.10	8032825.38	358.45	84.30
95.00	8146.80	8014821.84	357.64	84.11
160.00	7628.10	7504524.78	334.87	78.75
168.00	6000.40	5903193.52	263.42	61.93
184.50	7107.20	6992063.36	312.01	73.36
192.00	7122.90	7007509.02	312.70	73.52
208.00	6722.30	6613398.74	295.11	69.38
216.00	6977.50	6864464.50	306.31	72.02
234.00	6693.10	6584671.78	293.83	69.08
240.00	6869.00	6757722.20	301.55	70.90
256.50	6425.00	6320915.00	282.06	66.32

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APPENDIX E.

Reactor #3: Samples from NaOH trap (100 ul each)

Time	Corrected CPM	CPM per 30 ml	CPM/mg soil	% of starting CPM
0.00	0.00	0.00	0.00	0.00
16.50	55.70	16710.00	0.73	0.18
24.50	67.70	20310.00	0.89	0.22
40.50	167.10	50130.00	2.19	0.55
48.00	265.30	79590.00	3.48	0.87
64.50	428.50	128550.00	5.61	1.40
72.00	602.70	180810.00	7.90	1.98
88.00	1199.10	359730.00	15.71	3.93
95.00	1385.60	415680.00	18.15	4.54
160.00	4116.30	1234890.00	53.93	13.49
168.00	4609.70	1382910.00	60.39	15.11
184.50	4760.20	1428060.00	62.36	15.60
192.00	5219.60	1565880.00	68.38	17.11
208.00	5775.10	1732530.00	75.66	18.93
216.00	5305.90	1591770.00	69.51	17.39
234.00	5415.00	1624500.00	70.94	17.75
240.00	5289.20	1586760.00	69.29	17.33
256.50	5779.30	1733790.00	75.71	18.94

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APPENDIX F.

Reactor #3: Soil slurry samples (1 ml each)

Time	Corrected CPM	CPM per spl	CPM/mg soil	% of starting CPM
0.00	9165.80	9152051.30	399.65	100.00
16.50	8875.70	8862386.45	387.00	96.84
24.50	8665.10	8652102.35	377.82	94.54
40.50	8954.00	8940569.00	390.42	97.69
48.00	9081.00	9067378.50	395.96	99.08
64.50	8328.90	8316406.65	363.16	90.87
72.00	8637.20	8624244.20	376.60	94.23
88.00	7951.70	7939772.45	346.71	86.75
95.00	7885.90	7874071.15	343.85	86.04
160.00	7678.80	7667281.80	334.82	83.78
168.00	7006.10	6995590.85	305.48	76.42
184.50	6049.70	6040625.45	263.78	65.99
192.00	6774.00	6763839.00	295.36	73.89
208.00	6728.20	6718107.70	293.37	73.39
216.00	6785.10	6774922.35	295.85	74.01
234.00	6732.30	6722201.55	293.55	73.43
240.00	6778.10	6767932.85	295.54	73.93
256.50	6338.30	6328792.55	276.37	69.14